IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Chen et al. Art Unit :
Serial No. : Examiner :
Filed : February 20, 2002
Title : NOVEL MODIFIED MSP-1 NUCLEIC ACID SEQUENCES AND METHODS FOR INCREASING MRNA LEVELS AND PROTEIN EXPRESSION IN CELL SYSTEMS

Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Prior to examination, please amend the application as follows:

In the specification:

Insert the following paragraph at page 1, line 6:

-- This application is a continuation of U.S. Serial Number 09/175,684, filed October 20, 1998, which claims priority to U.S. Serial Number 60/085,649, filed May 15, 1998, and U.S. Serial Number 60/062,592, filed October 20, 1997, the contents of which are incorporated herein by reference. --

In the claims:

Cancel claims 1-8.

Add new claims 9-84 as follows:

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9. (New) A method of producing a merozite surface protein 1 (MSP-1) or a fragment thereof in the milk of a non-human transgenic mammal, comprising:

providing a non-human transgenic mammal whose genome comprises a modified nucleic acid encoding MSP-1 or fragment thereof operably linked to a promoter which directs expression in the mammary gland, wherein the nucleic acid has been modified by replacing at least one AT-containing codon of a wild-type nucleic acid sequence encoding MSP-1 with a preferred codon encoding the same amino acid as the replaced codon such that the AT-content of the modified nucleic acid is lowered as compared to the wild-type nucleic acid sequence encoding MSP-1; and allowing the transgenic mammal to express MSP-1 or fragment thereof in its milk, to thereby produce MSP-1 or fragment thereof.

10. (New) The method of claim 9, wherein the preferred codon is a mammary gland specific codon.

11. (New) The method of claim 10, wherein more than one codon in the wild-type nucleic acid sequence has been replaced with a preferred mammary gland-specific codon encoding the same amino acid as the replaced codon.

12. (New) The method of claim 10, wherein the AT content of the modified nucleic acid sequence has been reduced by replacing codons of the wild-type nucleic acid sequence with mammary gland specific codons encoding the same amino acid as the replaced codon such that the AT content of the modified nucleic acid is 50% or less.

13. (New) The method of claim 9, wherein the promoter is a beta casein promoter.

14. (New) The method of claim 9, wherein the wild type nucleic acid sequence has been altered such that at least one glycosylation site of MSP-1 is not functional.

15. (New) The method of claim 14, wherein the wild-type nucleic acid sequence has been altered such that all of the glycosylation sites of MSP-1 are not functional.

16. (New) The method of claim 9, wherein the wild type nucleic acid sequence which has been modified comprises the nucleic acid sequence of SEQ ID NO:2.

17. (New) The method of claim 16, wherein a glycosylation site at position 181 of the wild type MSP-1 amino acid sequence is altered such that it is not functional.

18. (New) The method of claim 16, wherein a glycosylation site at position 262 of the wild type MSP-1 amino acid sequence is altered such that it is non-functional.

19. (New) The method of claim 16, wherein glycosylation sites at positions 181 and 262 of the wild type MSP-1 amino acid sequence are altered such that they are not functional.

20. (New) A method of producing a merozite surface protein 1 (MSP-1) or fragment thereof in the milk of a non-human transgenic mammal, comprising:

providing a non-human transgenic mammal whose genome comprises a modified nucleic acid encoding MSP-1 or fragment thereof operably linked to a promoter which directs expression in the mammary gland, wherein the nucleic acid has been modified by replacing at least a portion of an mRNA instability motif in the coding sequence of a wild-type nucleic acid sequence encoding MSP-1 with a preferred codon encoding the same amino acid as the replaced codon; and

allowing the transgenic mammal to express MSP-1 or fragment thereof in its milk, to thereby produce MSP-1 or fragment thereof.

21. (New) The method of claim 20, wherein the preferred codon is a mammary gland specific codon.

22. (New) The method of claim 21, wherein more than one codon in the wild type nucleic acid sequence has been replaced with a preferred mammary gland-specific codon encoding the same amino acid as the replaced codon.

23. (New) The method of claim 21, wherein all of the mRNA instability motifs present in the wild type nucleic acid sequence encoding MSP-1 have been replaced with a preferred mammary gland specific codon encoding the same amino acid as the replaced codon.

24. (New) The method of claim 20, wherein the promoter is a beta casein promoter.

25. (New) The method of claim 20, wherein the wild-type nucleic acid sequence has been altered such that at least one glycosylation site of MSP-1 is not functional.

26. (New) The method of claim 25, wherein the wild-type nucleic acid sequence has been altered such that all of the glycosylation sites of MSP-1 are not functional.

27. (New) The method of claim 20, wherein the wild type nucleic acid sequence which has been modified comprises the nucleic acid sequence of SEQ ID NO:2.

28. (New) The method of claim 27, wherein a glycosylation site at position 181 of the wild type MSP-1 amino acid sequence is altered such that it is not functional.

29. (New) The method of claim 27, wherein a glycosylation site at position 262 of the wild type MSP-1 amino acid sequence is altered such that it is non-functional.

30. (New) A method for producing a merozite surface protein 1 (MSP-1) or fragment thereof in the milk of a non-human transgenic mammal, comprising:

providing a non-human transgenic mammal whose genome comprises a modified nucleic acid encoding MSP-1 or fragment thereof operably linked to a promoter which directs expression in the mammary gland, wherein the nucleic acid has been modified by

a) replacing at least a portion of an mRNA instability motif in the coding sequence of a wild type nucleic acid encoding MSP-1 with a preferred mammary gland-specific codon encoding the same amino acid as the replaced portion of the mRNA instability motif; and

b) replacing one or more AT-containing codons of the nucleic acid of the wild-type nucleic acid sequence with a preferred mammary gland-specific codon encoding the same amino acid as the replaced codon; and

allowing the transgenic mammal to express MSP-1 or fragment thereof in its milk, to thereby produce MSP-1 or fragment thereof.

31. (New) The method of claim 30, wherein the modified nucleic acid has the same codon of the wild-type nucleic acid replaced with a preferred mammary gland-specific codon such that both the AT content of the wild-type nucleic acid sequence is lowered and the mRNA instability motif of the wild-type nucleic acid is eliminated by the preferred mammary gland-specific codon.

32. (New) The method of claim 30, wherein all of the mRNA instability motifs present in the wild-type nucleic acid sequence have been replaced by a preferred mammary gland-specific codon.

33. (New) The method of claim 30, wherein the modified nucleic acid further comprises at least one additional codon other than the codon replaced to lower AT content or the codon replaced to eliminate an mRNA instability motif which has been replaced with a preferred mammary gland-specific codon.

34. (New) The method of claim 30, wherein all of the codons of the wild-type nucleic acid sequence have been replaced with a preferred mammary gland-specific codon.

35. (New) The method of claim 30, wherein the modified nucleic acid is expressed in milk at a level which is at least 25% more than the wild-type nucleic acid sequence is expressed under the same conditions.

36. (New) The method of claim 30, wherein the modified nucleic acid is expressed in milk at a level which is at least 50% more than the wild-type nucleic acid sequence is expressed under the same conditions.

37. (New) The method of claim 30, wherein the modified nucleic acid is expressed in milk at a level which is at least 100% more than the wild-type nucleic acid sequence is expressed under the same conditions.

38. (New) The method of claim 30, wherein all non-preferred mammary gland specific codons are replaced with preferred mammary gland specific codons.

39. (New) The method of claim 30, wherein the wild type nucleic acid sequence which has been modified comprises the nucleic acid sequence of SEQ ID NO:2.

40. (New) The method of claim 30, wherein a glycosylation site at position 181 of the wild type MSP-1 amino acid sequence is altered such that it is not functional.

41. (New) The method of claim 30, wherein a glycosylation site at position 262 of the wild type MSP-1 amino acid sequence is altered such that it is non-functional.

42. (New) A transgenic non-human mammal whose genome comprises a modified nucleic acid encoding MSP-1 or fragment thereof operably linked to a promoter which directs expression in the mammary gland, wherein the nucleic acid has been modified by replacing at least a portion of an mRNA instability motif in the coding sequence of a wild-type nucleic acid encoding MSP-1 with a preferred mammary gland-specific codon encoding the same amino acid as the replaced portion of the mRNA instability motif and replacing one or more AT-containing codons of the wild-type nucleic acid sequence with a preferred mammary gland-specific codon encoding the same amino acid as the replaced codon, wherein the transgenic mammal expresses MSP-1 or fragment thereof in its milk.

43. (New) The mammal of claim 42, wherein the modified nucleic acid has the same codon of the wild-type nucleic acid encoding MSP-1 replaced with a preferred mammary gland-specific codon such that both the AT content of the wild-type nucleic acid is lowered and the mRNA instability motif of the wild-type nucleic acid sequence is eliminated by the preferred mammary gland-specific codon.

44. (New) The mammal of claim 42, wherein all of the mRNA instability motifs present in the wild-type nucleic acid sequence have been replaced by a preferred mammary gland-specific codon.

45. (New) The mammal of claim 42, wherein the modified nucleic acid further comprises at least one additional codon other than the codon replaced to lower AT content or the codon replaced to eliminate an mRNA instability motif which has been replaced with a preferred mammary gland-specific codon.

46. (New) The mammal of claim 42, wherein all of the codons of the wild-type nucleic acid have been replaced with a preferred mammary gland-specific codon.

47. (New) The mammal of claim 42, wherein the modified nucleic acid is expressed in milk at a level which is at least 25% more than the naturally occurring nucleic acid is expressed under the same conditions.

48. (New) The mammal of claim 42, wherein the modified nucleic acid is expressed in milk at a level which is at least 50% more than the naturally occurring nucleic acid is expressed under the same conditions.

49. (New) The mammal of claim 42, wherein the modified nucleic acid is expressed in milk at a level which is at least 100% more than the naturally occurring nucleic acid is expressed under the same conditions.

50. (New) The mammal of claim 42, wherein all non-preferred mammary gland specific codons are replaced with preferred mammary gland specific codons.

51. (New) The mammal of claim 42, wherein the wild type nucleic acid sequence which has been modified comprises the nucleic acid sequence of SEQ ID NO:2.

52. (New) The mammal of claim 51, wherein a glycosylation site at position 181 of the wild type MSP-1 amino acid sequence is altered such that it is not functional.

53. (New) The mammal of claim 51, wherein a glycosylation site at position 262 of the wild type MSP-1 amino acid sequence is altered such that it is non-functional.

54. (New) The mammal of claim 42, wherein the promoter is a beta casein promoter.

55. (New) A transgenic non-human mammal whose genome comprises a modified nucleic acid encoding MSP-1 or fragment thereof operably linked to a promoter which directs expression in the mammary gland, wherein the nucleic acid has been modified by replacing at least one AT-containing codon of a wild-type nucleic acid sequence encoding MSP-1 with a preferred codon encoding the same amino acid as the replaced codon such that the AT-content of the modified nucleic acid is lowered as compared to the wild-type nucleic acid sequence encoding MSP-1, wherein the transgenic mammal expresses MSP-1 or fragment thereof in its milk.

56. (New) The mammal of claim 55, wherein the preferred codon is a mammary gland specific codon.

57. (New) The mammal of claim 56, wherein more than one codon in the wild-type nucleic acid sequence has been replaced with a preferred mammary gland-specific codon encoding the same amino acid as the replaced codon.

58. (New) The mammal of claim 56, wherein the AT content of the modified nucleic acid sequence has been reduced by replacing codons of the wild-type nucleic acid sequence with mammary gland specific codons encoding the same amino acid as the replaced codon such that the AT content of the modified nucleic acid is 50% or less.

59. (New) The mammal of claim 55, wherein the promoter is a beta casein promoter.

60. (New) The mammal of claim 55, wherein the wild type nucleic acid sequence has been altered such that at least one glycosylation site of MSP-1 is not functional.

61. (New) The mammal of claim 60, wherein the wild-type nucleic acid sequence has been altered such that all of the glycosylation sites of MSP-1 are not functional.

62. (New) The mammal of claim 55, wherein the wild type nucleic acid sequence which has been modified comprises the nucleic acid sequence of SEQ ID NO:2.

63. (New) The mammal of claim 62, wherein a glycosylation site at position 181 of the wild type MSP-1 amino acid sequence is altered such that it is not functional.

64. (New) The mammal of claim 62, wherein a glycosylation site at position 262 of the wild type MSP-1 amino acid sequence is altered such that it is non-functional.

65. (New) The mammal of claim 62, wherein glycosylation sites at positions 181 and 262 of the wild type MSP-1 amino acid sequence are altered such that they are not functional.

66. (New) A transgenic non-human mammal whose genome comprises a modified nucleic acid encoding MSP-1 or fragment thereof operably linked to a promoter which directs expression in the mammary gland, wherein the nucleic acid has been modified by replacing at least a portion of an mRNA instability motif in the coding sequence of a wild-type nucleic acid

sequence encoding MSP-1 with a preferred codon encoding the same amino acid as the replaced codon, wherein the transgenic mammal expresses MSP-1 or fragment thereof in its milk..

67. (New) The mammal of claim 66, wherein the preferred codon is a mammary gland specific codon.

68. (New) The mammal of claim 67, wherein more than one codon in the wild type nucleic acid sequence has been replaced with a preferred mammary gland-specific codon encoding the same amino acid as the replaced codon.

69. (New) The mammal of claim 67, wherein all of the mRNA instability motifs present in the wild type nucleic acid sequence encoding MSP-1 have been replaced with a preferred mammary gland specific codon encoding the same amino acid as the replaced codon.

70. (New) The mammal of claim 66, wherein the promoter is a beta casein promoter.

71. (New) The mammal of claim 66, wherein the wild-type nucleic acid sequence has been altered such that at least one glycosylation site of MSP-1 is not functional.

72. (New) The mammal of claim 71, wherein the wild-type nucleic acid sequence has been altered such that all of the glycosylation sites of MSP-1 are not functional.

73. (New) The mammal of claim 66, wherein the wild type nucleic acid sequence which has been modified comprises the nucleic acid sequence of SEQ ID NO:2.

74. (New) The mammal of claim 73, wherein a glycosylation site at position 181 of the wild type MSP-1 amino acid sequence is altered such that it is not functional.

75. (New) The mammal of claim 73, wherein a glycosylation site at position 262 of the wild type MSP-1 amino acid sequence is altered such that it is non-functional.

76. (New) The mammal of claim 73, wherein glycosylation sites at positions 181 and 262 of the wild type MSP-1 amino acid sequence are altered such that they are not functional.

77. (New) A merozite surface protein 1 (MSP-1) or fragment thereof produced by the method of claim 9.

78. (New) A merozite surface protein 1 (MSP-1) or fragment thereof produced by the method of claim 20.

79. (New) A merozite surface protein 1 (MSP-1) or fragment thereof produced by the method of claim 30.

80. (New) A merozite surface protein 1 (MSP-1) or fragment thereof produced by the method of claim 14.

81. (New) A merozite surface protein 1 (MSP-1) or fragment thereof produced by the method of claim 25.

82. (New) A merozite surface protein 1 (MSP-1) or fragment thereof produced by the method of claim 37.

83. (New) A merozite surface protein 1 (MSP-1) or fragment thereof having at least one amino acid which has been altered such that a glycosylation site is not functional.

84. (New) A merozite surface protein 1 (MSP-1) or fragment thereof having an amino acid sequence which has been altered such that all of the glycosylation sites are not functional.

REMARKS

Claims 9-84 are pending. Claims 1-8 have been cancelled.

Applicant asks that all claims be examined. Please apply any other charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: 8/20/02

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APPLICATION

FOR

UNITED STATES LETTERS PATENT

TITLE: NOVEL MODIFIED MSP-1 NUCLEIC ACID SEQUENCES AND METHODS FOR INCREASING MRNA LEVELS AND PROTEIN EXPRESSION IN CELL SYSTEMS

APPLICANT: LI HOW CHEN AND HARRY M. MEADE

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